Microenvironment of Adjacent Non-Neoplastic Regions Determines Prognostic Outcomes in Locally Advanced Colorectal Cancer after Surgical Resection: A Multi-Center & Multi-Omics study

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Study outline

1. Survival analysis

- Cell RNA sequencing (according to the mean score of the single sample gene set enrichment analysis (ssGSEA)) of the top 20 tumor DEGs set (tumor signature) in the normal tissue.
- We analyzed the differently expressed genes (DEGs) using RNA seq data between tumor and normal tissues and divided them into groups according to the mean score of the single sample gene set enrichment analysis (ssGSEA) of the top 20 tumor DEGs set (tumor signature) in the normal tissue.

- Patient with tumor-like normal microenvironment (NME): high level of tumor signature in normal tissue;
- Patient with healthy normal microenvironment (NME): low level of tumor signature in normal tissue.

- Then, we validated the prognostic role with TCGA cohort and analyzed differences between groups including 16S RNA sequencing and single-cell RNA sequencing (scRNA-seq) of tumor and normal tissues for biological interpretation.

2. Pathway analysis

- 16S RNA sequencing

- Comparison between subgroups

- Survival outcomes and pathway analysis

Conclusion

- Our study highlights that the normal tissue adjacent to a tumor could be a novel prognostic and survival marker for patients with localized CRC after surgical resection. Within a median follow-up of 58.2 months, the NME group showed poor 5-year recurrence-free survival (54.7 vs. 73.0%, HR = 1.94, P = 0.022) and 5-year overall survival (78.2 vs. 88.0%, HR = 1.76, P = 0.034).

- The survival and adverse biological processes, such as cell apoptosis, cell proliferation, cell migration, cell survival, and cell senescence, were upregulated in the NME group compared to the NME group.

- The NME group showed a higher proportion of EMP1+ cell clusters, which were related to bacterial invasion, leukocyte recruitment, inflammation, and epithelial-mesenchymal transition pathways. These Organized microenvironments with immune and epithelial cells may induce metachronous recurrence after surgical resection.

- Based on our classification, prudent dietary habits or more advanced treatment strategies may be required to improve survival outcomes of NME.

Spatial distribution of the microbiota

- Patient#1 was classified as NME, and 16S RNA gene sequencing revealed Treponema and Prevotella genera-dominant microbiota in the tumor and normal tissues. Bacterial colonies (bacteroides) were observed in both the tumor and normal mucosal areas. Patient#2 was classified as NME, whereas it was scarcely present in the normal tissue. The 16S RNA sequencing of the tumor revealed a Treponema genus-dominant microbiota, similar to that of patient#1, whereas 16S RNA sequencing of normal tissue showed abundant genera, such as Fasculatus/otimandius and Clostridiales genus.

Putative mechanism

- Microbiota dysbiosis may induce TSL7+ T cells, and TSL7+ T cells promote neutrophil retention, increasing crowds with CD4+ T cells, and inducing the production of high levels of GZMK, which in turn decreases c-cadherin in the epithelial membrane and promote tumor progression. A weak intestinal barrier may provoke more bacteria-infected cancer cells, promoting cancer cell progression via FMT and recruiting IL-17+ neutrophils and G8MN+CD8+ T cells, resulting in a vicious cycle.